

SEP 14 2005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. 09/365,349

Customer No.: 23379

Applicant: Terry et al.

Confirmation No. 1676

Filed: July 30, 1999

Group Art Unit: 1638

Docket No. B99-085

Examiner: Ibrahim, Medina

Title: *Heavy Metal Phytoremediation*

CERTIFICATE OF TRANSMISSION

I hereby certify that this comm is being transmitted by facsimile to the
Comm for Patent 571-273-8300 on September 14, 2005.

Signed


Richard Aron Osman

BRIEF ON APPEAL

The Honorable Board of Appeals and Interferences
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Honorable Board:

We appeal from the Jun 16, 2005 final rejection of claims 1-24.

REAL PARTY IN INTEREST

The real party in interest is The Regents of the University of California, the assignee of
this application.

RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related appeals or interferences.

STATUS OF CLAIMS

Claims 1-24 are rejected and subject to this appeal.

STATUS OF AMENDMENTS

All Amendments are believed to be properly before the Board; an after-final amendment
filed Jul 25, 2005 was entered by Advisory Action dated Jul 28, 2005.

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SUMMARY CLAIMED SUBJECT MATTER

The invention relates to a specific plant species (*Populus angustifolia*, *Nicotiana tabacum* or *Silene cucubalis*) that is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant. Specification, p.3, lines 19-20; p.6, lines 7-9.

GROUND OF REJECTION TO BE REVIEWED ON APPEAL

- I. WHETHER THE EXAMINER HAS PROPERLY REJECTED CLAIMS 1 and 10 UNDER 35USC112, first paragraph (enablement).

ARGUMENT

- I. THE EXAMINER HAS NOT PROPERLY REJECTED CLAIMS 1 and 10 UNDER 35USC112, first paragraph (enablement).

The previously appealed-from enablement rejection and the resultant Board Decision dated July 31, 2003 rely on Noctor et al.'s (1998 J Exp Bot 49, 623-647) reference to "preliminary experiments" wherein ECS-overexpressing poplars and non-transformed poplars accumulated Cd to a similar extent. The enablement rejection and Decision were expressly premised on an assumption: that "it would require an undue amount of experimentation to produce hyperaccumulating plants other than Brassica plants without further guidance from applicants as to why the construct produced a hyperaccumulating Brassica plant but failed to produce a hyperaccumulating poplar." Decision, p.14, lines 12-16.

As we explained in our prior Reply Brief, we do know why Noctor et al.'s early plants did not show hyper-accumulation:

Noctor et al. did not have the benefit of our Specification, which teaches how to make the claimed hyper-accumulating plants, including hyper-accumulating poplars. Noctor et al reports that in unpublished "preliminary experiments" they failed to obtain hyper-accumulating poplars. We do not know how Noctor et al. did their experiments, so it is not possible for us to determine why they failed: we do not know in what form they provided the Cd, we do not know whether their poplars were subject to other variables that would have interfered with accumulation, we do not know how they made their transformants, we do not know whether their

preliminary experiments were based on one or two anomalous plants, we do not know if their soil had other toxins or confounding microorganisms that may have independently depleted the supplemented Cd, etc. It is possible that the results of Noctor et al. are based on experimental error or contaminated materials. On the other hand, it is possible that they result merely from an insufficient sample size – had they generated sufficient data, they may well have obtained hyper-accumulators.

Reply Brief p.2, lines 13-25; also quoted in Decision, para. bridging p.11 and 12.

Indeed, the same laboratory later published their subsequent experiments (Arisi et al. 2000, *Physiol Plant* 109, 143-9, now of record). In these subsequent experiments, their ECS-overexpressing poplar did indeed provide higher cadmium accumulation than corresponding untransformed plants. (Arisi 2000; see abstract; para. bridging col. 1 and 2 of p.145; Fig.1). Of course, this subsequent report also had the benefit of the subject Applicant's intervening teachings, as reported in Zhu et al. *Plant Physiol* 119, 73-79, 1999 and Zhu et al., *Plant Physiol* 121, 1169-1177, as cited, *inter alia*, on p.144, col.1, lines 34-37 of Arisi 2000.

Arisi 2000 confirms that the disclosed methods produce a hyperaccumulating poplar as readily as they produce a hyperaccumulating Brassica plant. We attempted to provide this reference to the Board in a Request for Rehearing; however, the Board declined to consider evidence not previously made of record (Decision dated Sept 30, 2003, p.2, lines 5-8).

The enablement issue is whether the specification enables one of ordinary skill in the art to practice the invention as claimed without undue experimentation. Here, the product claims are drawn to a plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant. The corresponding method claims require only two steps (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a subject plant in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

The subject enablement rejection is limited to the scope of the recited plant. The claims require a plant structurally limited to a plant genetically engineered to overexpress glutamylcysteine synthetase and functionally limited to one which does in fact overexpress the

recited glutamylcysteine synthetase *and* thereby provides enhanced accumulation of the targeted heavy metal as compared with a corresponding wild type plant (see claim 1). The same claims, limited to Brassica plants, were allowed and issued in continuing application 09/933,549 (now US Patent No. 6,576,816). In an effort to expedite allowance, the pending claims have been limited to just three particularly preferred, alternative plant species: *Populus angustifolia*, *Nicotiana tabacum* and *Silene cucubalis*. Specification, p.6, lines 7-9. These are the same three plant species recited in Table 2, which was derived from an experimental research proposal to exemplify across a panel of defined alternative plant varieties and conditions the proof-of-principle experiments performed with Brassica plants and reported in the subsequent Experimental Protocols and Results section (p.8, line 20 - p.13, line 17). Note that these claims are narrowly drafted to specific plant *species*, whereas the issued patent encompasses all plant species of the Brassica *genus*.

The invention is premised on Applicants' finding that the recited glutamylcysteine synthetase effects heavy metal accumulation, is causative of heavy metal accumulation and is rate-limiting of heavy metal accumulation. The disclosure establishes a predictable relationship between heavy metal exposure and overexpression of glutamylcysteine synthetase; namely, that such overexpression promotes enhanced accumulation of the metal. Accordingly, the specification aptly enables one of ordinary skill in the art to practice the method in any plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provide enhanced accumulation of the heavy metal.

After our filing date, and citing publications describing our invention, Arisi 2000 reports successful heavy metal hyperaccumulation in a hybrid poplar overexpressing overexpress glutamylcysteine synthetase. Though not the particular poplar species of our claims, this study appears to undermine the rationale of the rejection, particularly as applied to claims 3 and 24 which are limited to a poplar species. Please note that the poplars of Arisi 2000 were newly generated in that 2000 study by micropropagation in vitro (Arisi 2000, p.144, para. bridging col.1 and col.2). The poplars of Arisi 1997 (Planta 203, 362-372) are not the same plants studied in Arisi 2000. Hence, the Action misstates the facts when it alleges that "the transformed plants of Arisi et al (1997) and Arisi (2000) are identical." Action p.6, last line. While the 2000 plants derived from previously produced clones, they are not the same plants, and were grown under different standards than the poplars of Arisi 1997.

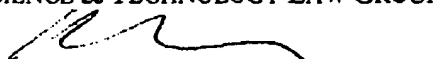
We are also unable to concur with the Action's proposal at p.7, lines 3 - 19 that reduced heavy metal tolerance is inconsistent with enhanced accumulation. In fact, these two properties often correlate, as discussed in the manuscript entitled "Expression of a High-Affinity Sulfate Transporter in *Brassica juncea* Affects Metal Tolerance and Accumulation" (Lindblom, Abdel-Ghany, Hanson, Hwang, Terry, and Pilon-Smits, 2005, submitted for publication, of record). In any event, our claims are indifferent to whether the plants display increased or decreased heavy metal tolerance.

For good measure, we have of record an expert declaration from a University of California Professor averring to the foregoing. The Declarant/Professor is knowledgeable of the dispositive factual determination of what one skilled in this art would and would not consider undue experimentation. This Declaration is authoritative evidence of a documented expert in the field of what the application enables one skilled in this art.

Appellants respectfully request reversal of the pending Final Action by the Board of Appeals.

The Appeal Brief Fee is provided in the attached PTO-2038. We petition for and authorize charging our Deposit Account No.19-0750 all necessary extensions of time. The Commissioner is authorized to charge any fees or credit any overcharges relating to this communication to our Dep. Acct. No.19-0750 (order B99-085).

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP



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EVIDENCE APPENDIX

The following Declaration of Professor Feldman under 37CFR1.132 was entered in the record with our Response filed Sep 20, 2004, and acknowledged by the Examiner in her Dec 22, 2004 Action at p.13, line 3.

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SEP 14 2005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Terry et al.

Group Art Unit: 1638

Serial No. 09/365,349

Examiner: Ibrahim, M.

Filed: July 30, 1999

For: *Heavy Metal Phytoremediation*

Attorney Docket No. B99-085

DECLARATION UNDER RULE 132

I, Lewis Feldman, declare and state as follows:

1. I am a Professor in the Department of Plant and Microbial Biology at the University of California, Berkeley. The Regents of the University of California is the assignee of the subject patent application. I am knowledgeable and experienced in the field of genetic engineering in plants. I have read and am familiar with the contents of the above application.

2. The product claims of this application are drawn to a plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant. The corresponding method claims require only two steps (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a subject plant in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

The specification teaches that "a wide variety of plants may be used, as urged by the particular trace element, medium, site geology, topology, weather, etc. Additional factors for selection include large biomass production, relatively high trace element accumulation capacity, and ease of genetic engineerability", citing Zhu et al., 1999, Plant Physiol 119:73-79. Specification, p.4, lines 6-9. The claims are structurally limited to a plant genetically engineered to overexpress glutamylcysteine synthetase and functionally limited to one which does in fact overexpress the recited glutamylcysteine synthetase and thereby provides enhanced accumulation of the targeted heavy metal as compared with a corresponding wild type plant (see

claim 1). "Suitable plants are readily screened for requisite engineerability and expression from exemplars of candidate plant varieties by those skilled in the art of plant genetic engineering, as exemplified below." Specification, p.4, lines 9-11. The specification offers a large number of suitable, commercially available varieties of exemplary plant source materials (p.4, line 11 - p.6, line 9). Furthermore, the specification describes diverse exemplary plant species demonstrating enhanced elemental assimilation in wild-type plants and the corresponding plant overexpressing a variety of recombinant glutamylcysteine synthetase genes (p.7, line 26 - p.8, line 18); exemplified plants include Brassica juncea, Populus angustifolia, Nicotiana tabacum and Silene cucubalis. The suitability of any given plant is readily ascertained by simple substitution into the same method.

The invention is premised on Applicants' finding that the recited glutamylcysteine synthetase effects heavy metal accumulation, is causative of heavy metal accumulation and is rate-limiting of heavy metal accumulation. The disclosure establishes a predictable relationship between heavy metal exposure and overexpression of glutamylcysteine synthetase; namely, that such overexpression promotes enhanced accumulation of the metal. This relationship is shown to hold across numerous and diverse exemplary plant species (supra). Accordingly, as an expert in the field, it is my opinion that the specification aptly enables one of ordinary skill in the art to practice the method in any plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provide enhanced accumulation of the heavy metal.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: 9/20/04


Prof. Lewis Feldman

RELATED PROCEEDINGS APPENDIX

No related proceedings are known to exist.

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CLAIMS APPENDIX

1. A plant which is a *Populus angustifolia*, *Nicotiana tabacum* or *Silene cucubalis* and is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant.
2. A plant according to claim 1 comprising a nucleic acid encoding the glutamylcysteine synthetase operably linked to a heterologous promoter.
3. A plant according to claim 1 which is a *Populus angustifolia*.
4. A plant according to claim 1 which is a *Nicotiana tabacum*.
5. A plant according to claim 1 wherein the heavy metal is selected from the group consisting of chromium, molybdenum and tungsten.
6. A plant according to claim 1 wherein the heavy metal is selected from the group consisting of cadmium and mercury.
7. A plant according to claim 1 wherein the heavy metal is uranium.
8. A plant according to claim 1, wherein the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.
9. A plant according to claim 1, wherein the plant comprises a nucleic acid encoding the glutamylcysteine synthetase operably linked to a heterologous promoter, the heavy metal is selected from the group consisting of chromium, molybdenum and tungsten and the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.
10. A plant according to claim 1, wherein the plant comprises a nucleic acid encoding the glutamylcysteine synthetase operably linked to a heterologous promoter, the heavy metal is selected from the group consisting of cadmium and mercury and the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.

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11. A plant according to claim 1, wherein the plant comprises a nucleic acid encoding the glutamylcysteine synthetase operably linked to a heterologous promoter, the heavy metal is selected from the group consisting of tellurium and polonium and the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.

12. A plant according to claim 1, wherein the plant comprises a nucleic acid encoding the glutamylcysteine synthetase operably linked to a heterologous promoter, the heavy metal is uranium and the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.

13. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 1 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

14. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 7 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

15. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 8 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

16. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 9 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

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17. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 10 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

18. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 11 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

19. A method according to claim 13, wherein the medium is soil.

20. A plant according to claim 1 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.

21. A plant according to claim 4 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.

22. A method according to claim 13 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.

23. A plant which is a commercially available variety of *Silene cucubalis* and is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant.

24. plant which is a commercially available variety of *Populus angustifolia* and is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant.

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